Pure

Scotland's Rural College

Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: a meta analysis

Cantalapiedra-Hijar, G; Dewhurst, RJ; Cheng, L; Cabrita, ARJ; Fonseca, AJM; Noziere, P; Makowski, D; Fouillet, H; Ortigues-Marty, I

Published in:
Animal

DOI:
10.1017/S1751731117003391

First published: 29/12/2017

Document Version
Peer reviewed version

Link to publication

Citation for pulished version (APA):
Cantalapiedra-Hijar, G., Dewhurst, RJ., Cheng, L., Cabrita, ARJ., Fonseca, AJM., Noziere, P., Makowski, D., Fouillet, H., & Ortigues-Marty, I. (2017). Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: a meta analysis. *Animal*, *12*(9), 1827 - 1837. https://doi.org/10.1017/S1751731117003391

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 04. May. 2023

Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: A meta-analysis

G. Cantalapiedra-Hijar[1], R.J. Dewhurst[2], L. Cheng[3], A.R.J. Cabrita[4], A.J.M. Fonseca[4], P. Nozière[1], D. Makowski[5], H. Fouillet[6] and I. Ortigues-Marty[1].

[1]Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès-Champanelle, France

[2]Scotland's Rural College, King's Buildings, West Mains Road, Edinburgh EH9 3JG, U.K.

[3]Faculty of Veterinary and Agricultural Sciences, Dookie Campus, Victoria 3647, The University of Melbourne, Australia

[4]REQUIMTE, LAQV, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal

[5]UMR Agronomie, INRA, AgroParisTech, Université Paris-Saclay, 78850 Thiverval-Grignon, France

[6]UMR Nutrition Physiology and Ingestive Behavior, AgroParisTech, INRA, Paris-Saclay University, F-75005 Paris, France

Corresponding author: Gonzalo Cantalapiedra-Hijar

Email: gonzalo.cantalapiedra@inra.fr

Short title: Prediction of nitrogen use efficiency in ruminants

Abstract:

Animal proteins are naturally ^{15}N enriched relative to the diet and the extent of this difference (**$\Delta^{15}N_{animal\text{-}diet}$** or N isotopic fractionation) has been correlated to N use efficiency (**NUE**; nitrogen gain or milk N yield/N intake) in some recent ruminant studies. The present study used meta-analysis to investigate whether $\Delta^{15}N_{animal\text{-}diet}$ can be used as a predictor of NUE across a range of dietary conditions, particularly at the level of between-animal variation. An additional objective was to identify variables related to N partitioning explaining the link between NUE and $\Delta^{15}N_{animal\text{-}diet}$. Individual values from 8 publications reporting both NUE and $\Delta^{15}N_{animal\text{-}diet}$ for domestic ruminants were used to create a database comprising 11 experimental studies, 41 treatments and individual animal values for NUE (n = 226) and $\Delta^{15}N_{animal\text{-}diet}$ (n = 291). Data were analyzed by mixed-effect regression analysis taking into account experimental factors as random effects on both the intercept and slope of the model. Diets were characterized according to the INRA feeding system in terms of N utilization at the rumen, digestive and metabolic levels. These variables were used in a Partial Least Squares regression analysis to predict separately NUE and $\Delta^{15}N_{animal\text{-}diet}$ variation, with the objective of identifying common variables linking NUE and $\Delta^{15}N_{animal\text{-}diet}$. For individuals reared under similar conditions (within-study) and at the same time (within-period), the variance of NUE and $\Delta^{15}N_{animal\text{-}diet}$ not explained by dietary treatments (i.e. between-animal variation plus experimental error) was 35% and 55% respectively. Mixed-effect regression analysis conducted with treatment means showed that $\Delta^{15}N_{animal\text{-}diet}$ was significantly and negatively correlated to NUE variation across diets ($NUE = 0.415 - 0.055 \times \Delta^{15}N_{animal\text{-}diet}$). When using individual values and taking into account the random effects of study, period and diet, the relationship was also significant ($NUE = 0.358 - 0.035 \times \Delta^{15}N_{animal\text{-}diet}$). However, there

may be a biased prediction for animals close to zero, or in negative, N balance. When using a novel statistical approach, attempting to regress between-animal variation in NUE on between-animal variation in $\Delta^{15}N_{animal\text{-}diet}$ (without the influence of experimental factors), the negative relationship was still significant, highlighting the ability of $\Delta^{15}N_{animal\text{-}diet}$ to capture individual variability. Among the studied variables related to N utilization, those concerning N efficiency use at the metabolic level contributed most to predict both $\Delta^{15}N_{animal\text{-}diet}$ and NUE variation, with rumen fermentation and digestion contributing to a lesser extent. This study confirmed that on average $\Delta^{15}N_{animal\text{-}diet}$ can predict NUE variation across diets and across individuals reared under similar conditions.

Keywords: ^{15}N, ruminant, nitrogen use efficiency, meta-analysis

Implications:

Variation in the N use efficiency in ruminants across diets, but also across individuals, can be predicted from the difference in the natural abundance of ^{15}N between the animal proteins and diet ($\Delta^{15}N_{animal\text{-}diet}$). The ability of this isotopic biomarker to rank individuals from a homogenous group (same animal species, physiological status and fed the same diet at the same time) could open the door to its application in genetic selection programs and precision livestock feeding.

Introduction

Improving the conversion of feed resources into animal products should be a goal in animal production systems aiming to solve future food security issues. Identifying feeding strategies, but also individual animals, leading to a greater efficiency of

nutrient utilization is therefore of crucial importance. Nitrogen use efficiency (**NUE**; nitrogen gain or milk N yield/N intake) is an important component of ruminant feed efficiency (Wheadon *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015) and logically determines the extent of N excretion to the environment. However, determination of NUE remains costly, laborious and difficult to accomplish under practical conditions. Accurate predictors of NUE are needed for precision management (feeding to individual potential), as well as for genetic selection. Previous studies have concluded that milk-N urea concentration in bulk tank milk (Kauffman and St-Pierre, 2001) or an average blood urea-N concentration (Kohn *et al.*, 2005) may be useful indicators of on-farm NUE. However, they appear less suitable for monitoring protein nutrition and N utilization of individual animals (Hof *et al.*, 1997; Huhtanen *et al.*, 2015), nor as a phenotyping tool to be applied in genetic selection for improved NUE (Vallimont *et al.*, 2010). An alternative and new biomarker for NUE in ruminants is based on the N isotopic fractionation between the animal and its diet (**$\Delta^{15}N_{animal\text{-}diet}$**, for details see Cantalapiedra-Hijar *et al.*, 2016). Variation in $\Delta^{15}N_{animal\text{-}diet}$ has a high potential to discriminate individuals fed the same diet but showing different N partitioning, as suggested by studies carried out in different animal species (Gaye-Siessegger *et al.*, 2004; Sears *et al.*, 2009; Warinner and Tuross, 2010) and humans (Fuller *et al.*, 2014). Several recent studies have evaluated this new biomarker of NUE in ruminants with promising results in most, although not all, cases (Cheng *et al.*, 2013; Cabrita *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015). The generalization of the relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ across different experimental conditions in ruminants has not yet been explored, nor has the potential of this new isotopic biomarker to predict between-animal variation in NUE.

Because ruminant N losses occur at both the rumen and animal metabolism levels (Dijkstra *et al.*, 2013), accurate biomarkers of NUE should be able to describe N partitioning at both levels. Although N isotopic fractionation in ruminants has been linked to both rumen bacterial (Sutoh *et al.*, 1993; Wattiaux and Reed, 1995) and splanchnic tissue (Cantalapiedra-Hijar *et al.*, 2015) metabolism, the significance of these pathways and biological mechanisms has only been evaluated under specific feeding conditions and with few animals (Cantalapiedra-Hijar *et al.*, 2016). Therefore, this study used meta-analysis to gauge the extent to which this isotopic biomarker can be proposed as a generalizable predictor of NUE variation – particularly at the level of between-animal variation (NUE variation in animals reared under identical conditions). An additional objective was to evaluate at which level (rumen, total gastrointestinal tract or metabolism) the N partitioning and utilization had a higher impact on the relationship between $\Delta^{15}N_{animal\text{-}diet}$ and NUE in ruminant animals under a range of feeding conditions.

Material and methods

Experimental data

Individual values from all available publications (Cheng *et al.*, 2011, 2013a, 2013b, 2014, 2016; Cabrita *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015, 2016) reporting both NUE and $\Delta^{15}N_{animal\text{-}diet}$ values in domestic ruminants were used to create a database ($n_{\_study}$ = 8). Overall, the database comprises 11 experimental studies (ID#1 to ID#11), 41 treatments (ID#_T) and individual animal values for NUE ($n_{\_indiv}$ = 226) and $\Delta^{15}N_{animal\text{-}diet}$ ($n_{\_milk}$ = 161 and $n_{\_plasma}$= 130) measured in dairy cattle ($n_{\_study}$ = 7), beef cattle ($n_{\_study}$ = 2), dairy goats ($n_{\_study}$ = 1) and non-lactating sheep ($n_{\_study}$ = 1). Trials were conducted as either factorial ($n_{\_study}$ = 3) or Latin square-like design

($n_{\_study}$ = 8). A description of the experimental studies and diets used in our database is available in the Supplementary material (Supplementary Tables S1 and S2).

Diet characterization and N utilization assessment

To evaluate at which level (rumen, total gastrointestinal tract or metabolism) the N utilization had a higher impact on the relationship between $\Delta^{15}N_{animal\text{-}diet}$ and NUE, experimental diets were characterized according to the new updated INRA feeding system (Sauvant and Nozière, 2016) and always using treatment mean values. Ingredients and the chemical composition of diets, average feed intake and average animal body weight from each dietary treatment were used to determine theoretical feed values, as well as theoretical digestive and metabolic variables related to N utilization through the systool software (www.systool.fr). The relationship between theoretical and measured dietary compositions did not differ from the first bisector with RMSE < 20 and 50 g/kg DM for CP and NDF, respectively. Diets were thus described in terms of rumen protein balance (**RPB**, g/kg DM), rumen degradable protein (**RDP**, g/kg DM), efficiency of microbial protein synthesis according to either available energy (**EMPS_E**, g/g rumen fermentable OM) or available protein (**EMPS_N**, g /g RDP), the digestive efficiency of N use (**DENU**, g metabolizable protein [PDI in the French system]/g crude protein) and the efficiency of MP utilization for either production (**EMPU_prod**, g of milk N secretion or N retention/g N from metabolizable protein intake) or for total net protein synthesis (**EMPU_tot**, [g of milk N secretion or N retention + endogenous fecal N + N lost in scurf]/[g N from metabolizable protein intake – g endogenous urinary N excretion]). These new variables were added to our database.

146 *Statistical analysis*

147 *Sources of variation in NUE and* $\Delta^{15}N_{animal\text{-}diet}$

148 We fitted a random intercept model, through the nlme package in the R
149 software (R Development Core Team, 2015), describing variability in NUE and
150 $\Delta^{15}N_{animal\text{-}diet}$, separately:

151

152 **(Eq. 1)** $Y_{ij} = \beta_0 + \beta_i + \varepsilon i_{j,}$

153

154 where, Y_{ij} is the observed NUE or $\Delta^{15}N_{animal\text{-}diet}$ values for observation j on the group i,
155 β_0 is the mean value across the population of domestic ruminants being sampled, β_i
156 is a random variable representing the deviation from the population mean for the i^{th}
157 group, and ε_{ij} is a random variable representing the deviation in NUE or $\Delta^{15}N_{animal\text{-}diet}$
158 values for observation j on group i from the mean value for group i. The grouping
159 factor (ID/P/D) included multiple nested levels of random effects for diet (D) within
160 experimental period (P) within study (ID). The residual error of this model
161 represented the between-animal variability together with the experimental error. Total
162 variance explained by the grouping factor was split to assess the proportion of
163 variance explained by each source of variation (ID, ID/P and ID/P/D).

164 Several parameters of interest were estimated with this approach, namely the
165 average values for NUE and $\Delta^{15}N_{animal\text{-}diet}$ for an "average" ruminant (β_0), the variance
166 of NUE and $\Delta^{15}N_{animal\text{-}diet}$ among experimental conditions (*between-group variability*
167 $[\sigma_i^2]$), and the variances of NUE and $\Delta^{15}N_{animal\text{-}diet}$ within the experimental unit (*within-*
168 *group variability* $[\sigma_e^2]$). With this random intercept model the intra-class correlation
169 coefficient (**ICC**) was calculated to assess the explained variance due to each level of
170 the nested grouping factor included in the model (ID, ID/P and ID/P/D). The ICC

gives an idea of the importance of the random variable (study, period and diet) to explain variation in NUE and $\Delta^{15}N_{animal\text{-}diet}$ values and it identified which grouping factors should be considered in the final mixed-effect model. The ICC was calculated as:

$ICC = \sigma^2 i / (\sigma_i^2 + \sigma_e^2)$

Analysis of the relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$

First, we used the lmList function of the nlme package (Pinheiro and Bates, 2000) to fit linear regressions relating NUE to $\Delta^{15}N_{animal\text{-}diet}$ for each study and dietary treatment separately (Figure 1). The confidence intervals were calculated for the individual regression coefficients and graphically presented (Figure 2) for evaluation of between-group variation. When there were indications of large study-to-study or diet-to-diet variation in either the intercept or slope estimates, a random effect was proposed in the model (Pinheiro and Bates, 2000).

Mixed-effect models were then used to test the ability of $\Delta^{15}N_{animal\text{-}diet}$ to reflect average NUE variation across diets and individuals. Different random structures (from simple to more complex models), were compared based on the Akaike Information Criterion (**AIC**; the lowest being best) and the Bayesian Information Criterion (**BIC**; the lowest being best). The random effects were tested on the intercept, slope or both. When information criterion statistics (AIC, BIC) were similar for two models, the log-likelihood ratio test criteria performed with the command ANOVA (model 0, model 1) in R was used to determine the best model. Random-effect structures were always compared (AIC, BIC) using the maximum likelihood method, while the coefficients of the final model were estimated using the restricted-maximum likelihood method.

196 The general form of the mixed-effect model was:

197 **(Eq. 2)** $Y_{ij} = (\beta_0 + b_{0i}) + (\beta_1 + b_{1i})X_{ij} + \varepsilon_{ij,}$

198

199 where Y_{ij} and X_{ij} are NUE and $\Delta^{15}N_{animal\text{-}diet}$ values, respectively, β_0 and β_1 are the
200 fixed effects for the intercept and the slope, respectively; the b_i are the random-
201 effects of experimental factors (the effect of study when using mean treatment values
202 and the effect of either study, period within-study or diet within-period and study when
203 using individual values) and assumed to be independent for different factors; and $\varepsilon_{ij,}$
204 are the identically distributed within-groups errors, assumed to be independent of the
205 random effects. Because of the relative small number of data and complex random-
206 effect structure, a diagonal variance-covariance structure for the random effects was
207 chosen to obtain convergence for the most complex models (Pinheiro and Bates,
208 2000).

209 An alternative approach, inspired from Phuong *et al.* (2013), was adopted to
210 attempt to regress between-animal variation in NUE against between-animal variation
211 in $\Delta^{15}N_{animal\text{-}diet}$ regardless of the influences of experimental factors. The between-
212 animal variability in both NUE and $\Delta^{15}N_{animal\text{-}diet}$ was approached once the random
213 effect of the experimental study (ID), period within-study (ID/P) and diet within-period
214 and study (ID/P/D]) were removed from raw values according to Eq1. The resulting
215 residuals for NUE and $\Delta^{15}N_{animal\text{-}diet}$ (Table 1) were considered mainly due to the
216 between-animal variation and unidentified sources of error (within-animal variation). If
217 a relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ was still significant once the raw values
218 were devoid of the influence of experimental factors, the ability of this biomarker to
219 capture between-animal variation in NUE would be proven.

220

Identification of outliers

A two-sided outlier test for the standardized residuals was performed at every step to identify outlier observations. Observations with absolute standardized residuals (random effects) greater than the 1−(0.05/2) quantile of the standard normal distribution were thus identified (Pinheiro and Bates, 2000). Outliers were only removed from the database if biological reasons justified their elimination.

Partial Least Square regression analysis

To answer the question about which variables related to N utilization could better explain the link between $\Delta^{15}N_{animal\text{-}diet}$ and NUE, a partial least-squares (**PLS**) regression analysis was carried out (XLStat v2015.2.02), independently for both variables, on descriptors of N use at the rumen, digestive and metabolic levels. PLS analysis circumvents the problem of multicollinearity between variables related to N partitioning. Thus, the PLS model included either NUE or $\Delta^{15}N_{animal\text{-}diet}$ as the dependent variable and the 7 descriptors of N partitioning as independent variables (RPB, RPD, EMPS_E, EMPS_N, DENU, EMPU_prod and EMPU_tot). To determine the number of components to keep in the PLS model, the cross-validation criterion Q2 was considered (Q2 > 0.0975). The importance of each variable in the model was assessed through their variable importance in projection (VIP) scores. Variables with VIP scores higher than one are considered most important.

Results

Description of the meta-design

Three diets out of 41 (nine individual NUE observations out of 226 [4.0%]) belonging to the same study (ID#7) were removed from our database based on both statistical

(large standardized residuals) and biological reasons as discussed later. The number of individual observations within each study ranged from 15 to 34 (Supplementary Table S1) and there were from 3 to 16 observations for each dietary treatment. Most studies (8 out of 11) tested the dietary treatments across several experimental periods (either 3 or 4) and in these cases each diet was tested on either 1 (ID#2, ID#3, ID#7) or 2 (ID#8 and ID#11) or 3 (ID#4, ID#5 and ID#6) animals per period. As shown in Supplementary Table S1, the 11 experiments cover a large range of NUE values (from -0.140 to 0.394 g/g) and show a high variability (CV = 40%) in relation to its mean value (0.243 g/g). This variability in NUE resulted from the heterogeneity of experimental studies in terms of type of ruminant, experimental diets (Supplementary Table S2; CP content ranging from 128 to 268 g/kg DM and NE content from 1.16 to 1.99 Mcal/kg DM) and feeding level (Supplementary Table S2). The difference in natural ^{15}N abundance between the ruminant and its diet ($\Delta^{15}N_{animal\text{-}diet}$) averaged 3.28‰ (CV = 41%) and ranged from 1.01 to 5.70‰ across diets and studies. Four studies reported $\Delta^{15}N_{animal\text{-}diet}$ values both in plasma and milk proteins, but no effect of the type of sample (plasma vs milk) was noted either on the intercept (*P = 0.28*) or on the slope (*P = 0.39*) from the overall relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ across these four studies (Supplementary Figure S1). Thus, all $\Delta^{15}N_{animal\text{-}diet}$ data ($n_{\_milk}$ = 152 and $n_{\_plasma}$ = 130) were used in subsequent regression analysis to improve model predictions.

Identified sources of variation

Variance components estimates (Table 1) calculated through a random intercept model showed the effect of study as a strong grouping factor explaining 76% and 85% of the total variance (intra-class correlation coefficients) of NUE and

271 $\Delta^{15}N_{animal\text{-}diet}$, respectively. This means that around three quarters of variation
272 observed in values for our dependent variable (NUE) was explained by variation
273 among studies (between-study variability). The random effect of period (time) further
274 explained one third of the remaining NUE and $\Delta^{15}N_{animal\text{-}diet}$ variation. For this part,
275 within a given study and experimental period the random effect of the diet explained
276 around 65% and 45% of NUE and $\Delta^{15}N_{animal\text{-}diet}$ variation, respectively.
277
278 *Relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$*
279 Figure 1 shows the relationship between NUE and N isotopic fractionation
280 when all individual data ($n_{\_indiv}$ = 217) were regressed using a simple linear
281 regression (between-study regression in Fig. 1a), or when individual linear fits were
282 obtained for each study (within-study regression in Fig. 1b; $n_{\_study}$ = 11) or diet
283 (within-diet regression in Fig. 1c; $n_{\_trt}$ = 38). A significant ($P < 0.001$) quadratic term
284 for $\Delta^{15}N_{animal\text{-}diet}$ was noted when conducting the between-study regression analysis
285 (Fig. 1a), slightly improving the fit (R^2 = 0.75; data not shown).
286 Although the response of NUE to $\Delta^{15}N_{animal\text{-}diet}$ variation (slope) was always
287 negative within-study (Figure 2a) only 6 out of 11 were significantly ($P < 0.05$)
288 different from 0. Likewise, although most (31 out of 38) slopes were negative within-
289 diet (Figure 2b) only around 29% (9 out of 31) were significantly ($P < 0.05$) different
290 from 0. The confidence intervals for the intercepts and slopes across studies and
291 diets did not always overlap; with the most negative slope and highest intercept for
292 the only study conducted with non-productive animals (study ID#8). A high variability
293 among studies and diets was thus evidenced, suggesting the need for different
294 intercepts and response (slope) coefficients among experimental conditions in our
295 model.

296 Regardless of the approach (treatment means vs individual values) and based
297 on AIC/BIC criteria, as well as on the comparison of random variance structure
298 through the likelihood ratio test, the best mixed-model included the random effects of
299 experimental factors on both the intercept and slope (Table 2); that is the most
300 complex model structure. Based on variance component estimates of the best mixed-
301 effect model using individual values, it is concluded that the influence of experimental
302 factors (study, period and diet) was higher for variation in NUE response to $\Delta^{15}N_{animal-diet}$ (σ = 0.010, 0.005 and 0.007 for an average slope of -0.035 [14%<CV<29%]) than
304 for mean estimates of NUE (σ = 0.024, 0.0000019, 0.020 for an average intercept of
305 0.358 [CV < 7%]).

306 On average, the relationship between individual values of NUE and $\Delta^{15}N_{animal-diet}$ adjusted by the random effects of the study, period (within-study) and diet (within-
308 period and study) had a significant and negative slope of -0.035 g/g (Figure 3) - much
309 less pronounced than that obtained for the unadjusted between-study regression
310 analysis (Figure 1; -0.058 g/g) or when the analysis was based on treatment means
311 and corrected for the effect of study (Table 2; -0.055 g/g). However, there may be a
312 biased prediction for ruminants fed close to maintenance requirements (ID#8; black
313 circles in Figure 4).

314 Finally, when individual data for NUE and $\Delta^{15}N_{animal-diet}$ were independently
315 adjusted by the random effects of the study, period (within-study) and diet (within-
316 period and study), their residuals, mainly representing the between-animal variation,
317 were still negatively correlated with each other (P < 0.001) though with a poor fit (r^2 =
318 0.12; Figure 4).

319

320 *N partitioning and N isotopic fractionation*

Relationships between $\Delta^{15}N_{animal\text{-}diet}$ and variables related to N use at the whole body, rumen, digestive and metabolic levels are presented in Table 3. Overall, $\Delta^{15}N_{animal\text{-}diet}$ showed a high degree of correlation with most variables, with coefficients of determination (r^2) ranging from 0.30 (EMPS_E) to 0.83 (NUE). The $\Delta^{15}N_{animal\text{-}diet}$ was negatively correlated to variables reflecting the efficiency of N utilization at the different levels (EMPU_prod, EMPU_tot, DENU, EMPS_E and EMPS_N) while positively correlated to variables reflecting protein degradation in the rumen (RDP and RPB). Under the experimental conditions of the studies used in this meta-analysis and according to dietary characterization by the INRA feeding system, the measured whole-body NUE showed a higher correlation with the efficiency of metabolic N use (r = 0.87 and 0.80 for EMPU_prod and EMPU_tot, respectively) compared with variables reflecting the efficiency of N use in the rumen (r = 0.53 and 0.43 for EMPS_E and EMPS_N, respectively) or digestive (r = 0.61 for DENU) levels.

PLS regression models were developed independently to predict variation in $\Delta^{15}N_{animal\text{-}diet}$ or NUE as a function of variables related to N utilization (Figure 5). In both cases, the best PLS regression models contained three components. These components explained 96% and 87.0% of the observed $\Delta^{15}N_{animal\text{-}diet}$ variation (R2Y) and could predict 93% and 83.0% of variation in new values (predictive ability of the model; Q2), respectively. The error of prediction of the model was relatively low (MSEP = 0.017 and 0.44 for NUE and $\Delta^{15}N_{animal\text{-}diet}$, respectively) compared to the range of observed values (Supplementary Table S1). Metabolic N use efficiencies (EMPU_tot and EMPU_prod) were the most important variables in both predictive models as evidenced by their VIP values (from 1.20 to 1.41 for ENU and from 1.12 to 1.25 for ENU and $\Delta^{15}N_{animal\text{-}diet}$, respectively). In both cases, the VIP scores for the other variables were lower than the unity for all components. However, when the PLS

346 model for $\Delta^{15}N_{animal\text{-}diet}$ was constructed using only these two variables the resulting
347 predictive performances were much lower (data not shown), highlighting the
348 importance of all variables for explaining variation in N isotopic fractionation.
349
350 **Discussion**
351 High between-animal variability in $\Delta^{15}N_{animal\text{-}diet}$ has been reported for
352 ruminants fed the same diet and reared under the same conditions (Hartman, 2011;
353 Sponheimer *et al.* 2003). Within our database, we noted that variation in $\Delta^{15}N_{animal\text{-}diet}$
354 for animals reared under identical conditions (same species, study, diet and period)
355 was as large as 1.7‰ (data not shown), which is half of the accepted trophic shift
356 value (3.4‰; Minagawa and Wada, 1984), that is the threshold which ecologists use
357 to distinguish trophic levels. Between-individual variation in $\Delta^{15}N_{animal\text{-}diet}$ could stem
358 from individual differences in protein balance (Sick *et al.*, 1997; Fuller *et al.*, 2004). In
359 this sense, one of the first authors to evoke the potential link between NUE and
360 $\Delta^{15}N_{animal\text{-}diet}$ was Vanderklift and Ponsar (2003). Since then different studies
361 proposed or confirmed a strong relationship between the N isotopic fractionation and
362 NUE in fish (Gaye-Siessegger *et al.*, 2004), birds (Sears *et al.*, 2009), pigs (Warinner
363 and Tuross, 2010) and ruminants (Cheng *et al.*, 2014; Cantalapiedra-Hijar *et al.*,
364 2015). In the present study, we explored by meta-analysis the relationship between
365 $\Delta^{15}N_{animal\text{-}diet}$ and NUE in domestic ruminants through different statistical approaches
366 and found on average a significant and negative correlation between them, even at
367 the level of between-animal variation (Table 2 and Figures 3 and 4).
368
369 *Data exclusion from the database*

Three dietary treatments belonging to the same study (Cheng *et al.*, 2011) were first identified as outliers and excluded from our database based on biological reasons. They had a particular chemical composition (forages with high ammonia-N content [almost 15% of total N, on average] two fold higher compared to the other 6 experimental diets from the same study) and were associated with high standardized residuals when exploring the overall and within-study relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$. One possible explanation for the very low $\Delta^{15}N_{animal\text{-}diet}$ values observed for these three treatments (ranging from 0.81 to 2.06) compared to the rest of diets (ranging from 2.33 to 3.63) could be differences in N isotopic fractionation by rumen bacteria depending on the nature of the N source (ammonia vs amino acids; Wattiaux and Reed, 1997). When data from these three diets were removed a negative rather than positive (Cheng *et al.* 2011) relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ was found (ID#7 in Figure 2) in accordance with the average trend of other studies in our database (Cheng *et al.*, 2014; Cantalapiedra-Hijar *et al.*, 2015). Likewise, a positive rather than negative relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ can be calculated from the N balance and isotopic data reported by Sutoh *et al.* (1993) in sheep receiving diets based on alfalfa hay supplemented or not with sucrose. A different pattern of N isotopic fractionation at the rumen level when animals are fed diets rich in rumen degradable protein, as it is the case with alfalfa hay, cannot be excluded and could agree with the unexpected positive relationship found for three high ammonia diets removed from our database as discussed above. In the present meta-analysis, rumen protein balance and rumen degradable protein, both of them associated with rumen ammonia-N concentration, were moderately and positively correlated (0.63-0.64) to $\Delta^{15}N_{animal\text{-}diet}$, but they were not, however, the most important variables explaining $\Delta^{15}N_{animal\text{-}diet}$ variation in the PLS analysis (Figure 5). Further

study is needed to better understand the role of rumen metabolism in N isotope fractionation when there is a large excess of rumen degradable protein.

Identifying main sources of variation

The relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ might not be unique across experimental conditions: the confidence intervals for the estimated slope did not always overlap across studies and diets. Nevertheless, it is not possible to accurately predict this relationship for individual diets since the low number of replications led to inaccurate estimates; that is large confidence intervals as shown in Figure 2. Indeed, despite a negative response of NUE to variation in $\Delta^{15}N_{animal\text{-}diet}$ for most diets, few significant coefficients were found (9 out of 38). The use of a mixed-effect regression analysis in our meta-analysis allowed us to estimate an average trend between NUE and $\Delta^{15}N_{animal\text{-}diet}$ without the need to accept that the relationship might be different for each experimental condition. The need to account for the random effects of known experimental conditions was thus graphically confirmed and agreed with the previous intra-class correlation analysis (Table 1) showing the effect of study as a very high grouping-factor.

Despite some results suggesting lower $\delta^{15}N$ values in milk vs plasma samples within the same individual (Jenkins *et al.,* 2001), no effect of sample type was noted in the overall relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ in the present study (Supplementary figure S1). Finally, because inter-species variation may exist in rumen and metabolic N utilization, the relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ might differ across ruminant species (cattle, goat and sheep). Unfortunately, there were too few data available in the literature to address this question.

420 *Mixed-effect analysis based on treatment means*

421 On average, a global and significant negative relationship between NUE and
422 $\Delta^{15}N_{animal\text{-}diet}$ was found under the main influence of dietary treatments (Eq.3 in Table
423 2; slope = -0.055). The strong impact of diet quality, mainly the protein content and
424 the nature of the protein, on $\Delta^{15}N_{animal\text{-}diet}$ variation is well documented in the literature
425 (Poupin *et al.*, 2011; Vanderklift and Ponsar, 2003). Likewise, dietary protein content
426 (Huhtanen and Hristov, 2009) and quality (rumen degradable vs undegradable
427 protein; Hristov *et al.*, 2004) are known to be two of the main determinants of NUE in
428 ruminants. The slope found with this approach suggests that on average an increase
429 in $\Delta^{15}N_{animal\text{-}diet}$ of 1‰ observed between two dietary treatments is associated with a
430 0.055 g/g decrease in NUE. This negative slope is compatible with a difference in
431 $\Delta^{15}N_{animal\text{-}diet}$ of around 1.5‰ reported by Sponheimer *et al.* (2003a) in cattle fed two
432 contrasting diets and leading to an estimated difference in NUE (calculated from N
433 balance) of 0.056 g/g in llamas subsequently fed the same diets (Sponheimer *et al.*,
434 2003b).

435

436 *Mixed-effect analysis based on individual values*

437 On average, we found a significant and negative relationship between
438 individual values of NUE and $\Delta^{15}N_{animal\text{-}diet}$. The discrepancy between the slope
439 obtained when using individual (-0.035 g/g) rather than treatment mean (-0.055 g/g)
440 values could stem from the smaller influence, but still significant, of between-animal
441 variation in the relationship between NUE and $\Delta^{15}N_{animal\text{-}diet}$ compared to other
442 experimental conditions such as physiological animal status or dietary treatments. An
443 alternative explanation could be that physiological mechanisms explaining between-

444 animal variation in NUE are not exactly the same as those related to between-diet
445 variation in NUE.

446 Indeed the variance estimates calculated from a simple intercept model
447 showed the effect of the study as a strong grouping variable explaining more than
448 half of total variance in NUE and in agreement with previous studies (Kohn *et al.*,
449 2005; Huhtanen *et al.*, 2015). The high diversity of experimental conditions included
450 in our database in terms of animal species, physiological status and experimental
451 diets explained the large contribution of study to NUE variation. However, within a
452 given study and experimental period it is noted that the variance unexplained by the
453 diet, and corresponding mainly to between-animal variation, was still very high (35%
454 and 55% of the NUE and $\Delta^{15}N_{animal\text{-}diet}$ variation, respectively). Thus, by applying a
455 mixed-effect model to our individual data to take into account the random effects of
456 the study, diet and period we demonstrated that on average $\Delta^{15}N_{animal\text{-}diet}$ is
457 negatively related to between-animal variation in NUE, which otherwise would have
458 been only evident for 9 out of 38 diets (Figure 2).

459

460 *Limits of the prediction model*

461 A generalizable equation might result in a biased estimation of NUE,
462 especially when animals are in a negative or close to zero N balance as it is the case
463 for data from the only study carried out on adult ruminants fed close to maintenance
464 energy requirements (ID#8 represented as black circles in Figure 3). Data from that
465 study seem to be responsible for a slightly better fitting when a quadratic term was
466 included in the unadjusted between-study relationship of NUE vs $\Delta^{15}N_{animal\text{-}diet}$ (Figure
467 1a; data not shown). The sharper slope in the relationship between NUE and
468 $\Delta^{15}N_{animal\text{-}diet}$ found in the study ID#8 (Figure 2) supports the concept of a higher ^{15}N

enrichment over the diet when the main protein source for the individual is its own stores (Martinez del Rio *et al.*, 2009) as found for example when birds lose weight during egg-laying and incubation (Hobson *et al.*, 1993), when feeding levels were below maintenance requirements in fish (Gaye-Siessegger *et al.*, 2007) or when pregnant women lose weight during nutritional stress (Fuller *et al.*, 2014). However, we contend that this does not preclude the possibility of using $\Delta^{15}N_{animal\text{-}diet}$ to rank such animals for NUE. Future studies should investigate effects of body N reserve mobilization on relationships with $\Delta^{15}N_{animal\text{-}diet}$.

Mixed-effect regression based on residuals

A difference in $\Delta^{15}N_{animal\text{-}diet}$ values of 1‰ for animals fed the same diet at the same time (and site) was associated on average with a significant difference of 0.024 g/g in NUE, which represent around 10% of the mean value of NUE obtained in our database (Supplementary Table S1). This result highlights the ability to use this new isotopic biomarker to rank animals reared under similar conditions for NUE, although with a greater uncertainty than for diets, and it agrees with results obtained by Gaye-Siessegger *et al.* (2004) in 32 fish showing large between-animal variation in NUE (from around -0.05 to around 0.40 g/g). Our finding also agrees with the significant and negative relationship found by Wheadon *et al.* (2014) when $\Delta^{15}N_{animal\text{-}diet}$ was used as a predictor of between-animal variation in feed conversion efficiency (average daily gain/dry matter intake) in 84 growing beef heifers fed the same diet at the same time (slope = -0.014 g/g FCE). The relatively sharper slope found in the present study compared to that found by Wheadon *et al.* (2014) could be explained by the fact that lipids contribute to body weight gain, and therefore to feed conversion efficiency, but not to NUE.

494

495 *Link between N partitioning and N isotopic fractionation*

496 In the present study, the two variables related to the N use efficiency at the
497 metabolic level (EMPU_tot and EMPU_prod) were the most important parameters,
498 based on their VIP values, explaining both NUE and $\Delta^{15}N_{animal\text{-}diet}$ variation. These
499 results agree with two studies conducted in rats (Sick *et al.*,1997; Poupin *et al.*, 2014)
500 and showing that the $\Delta^{15}N_{animal\text{-}diet}$ correlated well with the balance between protein
501 synthesis vs catabolism in the liver, an indicator of metabolic N efficiency use.
502 Although there is clear evidence of a N isotopic fractionation carried out by rumen
503 bacteria (Sutoh *et al.*, 1993; Wattiaux and Reed, 1995) our findings suggests that on
504 average they would contribute to a lesser extent to $\Delta^{15}N_{animal\text{-}diet}$ variation than
505 metabolic processes. This result agrees with the concept of a higher contribution of
506 animal metabolism to between-animal variation in feed efficiency compared to other
507 determinants such as digestion, heat increment of feeding or feeding pattern
508 (Richardson and Herd, 2004). This is likely one of the main reason why milk urea-N
509 concentration may be unable to capture properly between-animal variation in NUE in
510 ruminants (Vallimont *et al.*, 2011; Huhtanen *et al.* 2015), since their values have been
511 mostly correlated to rumen N losses (Nouisiainen *et al.*, 2004, Hof *et al.*, 1997) rather
512 than to the efficiency of metabolic N use (Hof *et al.*, 1997).

513 Our results may suggest that the ability of $\Delta^{15}N_{animal\text{-}diet}$ to describe the
514 between-animal variation in NUE can be due to the strong link between the N isotopic
515 fractionation and metabolic use of N. Finally, one strength of $\Delta^{15}N_{animal\text{-}diet}$ is the
516 virtually lack of diurnal variation and its stability irrespective of the feeding time given
517 its slow turnover rate. This can be advantageous when seeking to predict NUE in
518 animals adapted to their diets over long periods, but showing different daily feeding

patterns. In contrast, the period of time between the introduction of a new diet and the blood/milk sampling is an issue when predicting NUE through $\Delta^{15}N_{animal\text{-}diet}$ values, as revealed in our meta-analysis (the effect of period as a grouping-factor), since it takes a long time to reach a new isotopic equilibrium (proposed lag of 45 days for plasma proteins in ruminants, Cantalapiedra-Hijar *et al.*, 2015). Future research should evaluate the complementarity between MUN and $\Delta^{15}N_{animal\text{-}diet}$ and explore the natural abundance of ^{15}N in specific N compounds (such as individual amino acids) to better predict NUE.

Conclusions

We showed through a meta-analysis approach that the natural ^{15}N enrichment of ruminant protein over the consumed diet ($\Delta^{15}N_{animal\text{-}diet}$) is on average significantly and negatively correlated to N use efficiency in domestic ruminants reared under different conditions. This correlation was significant even at the between-animal level, highlighting the ability of this isotopic biomarker to rank individuals from a homogenous group (same animal species, physiological status and fed the same diet at the same time) in terms of N use efficiency. The most important variables related to N utilization explaining the link between $\Delta^{15}N_{animal\text{-}diet}$ and NUE under the studied conditions were those related to N metabolism, with rumen fermentation and digestion contributing to a lesser extent.

Acknowledgements

The authors thank the many people involved in each experiment used in this meta-analysis study.

References

Cabrita ARJ, Fonseca AJM, and Dewhurst RJ 2014. Short communication: Relationship between the efficiency of utilization of feed nitrogen and 15 N enrichment in casein from lactating dairy cows. Journal of Dairy Science 97, 7225-7229.

Cantalapiedra-Hijar G, Ortigues-Marty I, Sepchat B, Agabriel J, Huneau JF, and Fouillet H 2015. Diet–animal fractionation of nitrogen stable isotopes reflects the efficiency of nitrogen assimilation in ruminants. British Journal of Nutrition 113, 1158-1169.

Cantalapiedra-Hijar G, Fouillet H, Huneau JF, Fanchone A, Doreau M, Nozière P, and Ortigues-Marty I 2016. Relationship between efficiency of nitrogen utilization and isotopic nitrogen fractionation in dairy cows: contribution of digestion v. metabolism? Animal 10, 221-229.

Cheng L, Kim EJ, Merry RJ, and Dewhurst RJ 2011. Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. Journal of Dairy Science 94, 2031-2041.

Cheng L, Sheahan AJ, Gibbs SJ, Rius AG, Kay JK, Meier S, Edwards R, Dewhurst RJ, and Roche JR 2013. Technical note: nitrogen isotopic fractionation can be used to predict nitrogen-use efficiency in dairy cows fed temperate pasture. Journal of Animal Science 91, 5785-5788.

Cheng L, Nicol AM, Dewhurst RJ, and Edwards GR 2013. The effects of dietary nitrogen to water-soluble carbohydrate ratio on isotopic fractionation and partitioning of nitrogen in non-lactating sheep. Animal 7, 1274-1279.

Cheng L, Woodward SL, Dewhurst RJ, Zhou H, and Edwards GR 2014. Nitrogen partitioning, energy use efficiency and isotopic fractionation measurements from cows differing in genetic merit fed low-quality pasture in late lactation. Animal Production Science 54, 1651-1656.

Cheng L, Edwards GR, Dewhurst RJ, Nicol AM, and Pacheco D 2016. The effect of dietary water soluble carbohydrate to nitrogen ratio on nitrogen partitioning and isotopic fractionation of lactating goats offered a high-nitrogen diet. Animal 10, 779-785.

571 Dijkstra J, Reynolds CK, Kebreab E, Bannink A, Ellis JL, France J, and Van Vuuren AM
572 2013. Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. In Energy
573 and Protein Metabolism and Nutrition in Sustainable Animal Production (pp. 47-58).
574 Wageningen: Wageningen Academic Publishers.
575 Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, and Hedges RE 2004. Nitrogen
576 balance and δ15N: why you're not what you eat during pregnancy. Rapid Communications in
577 Mass Spectrometry18, 2889-2896.
578 Gaye-Siessegger J, Focken U, Muetzel S, Abe, H, and Becker K 2004. Feeding level and
579 individual metabolic rate affect δ13C and δ15N values in carp: implications for food web
580 studies. Oecologia 138, 175-183.
581 Gaye-Siessegger J, Focken U, Abe, HJ, and Becker K 2007. Starvation and low feeding
582 levels result in an enrichment of 13C in lipids and 15N in protein of Nile tilapia Oreochromis
583 niloticus L. Journal of Fish Biology 71, 90-100.
584 Hartman G 2011. Are elevated δ15N values in herbivores in hot and arid environments
585 caused by diet or animal physiology?. Functional Ecology 25, 122-131.
586 Hobson KA, Alisauskas RT, and Clark RG 1993. Stable-nitrogen isotope enrichment in avian
587 tissues due to fasting and nutritional stress: implications for isotopic analyses of diet.
588 The Condor 95, 388-394.
589 Hof G, Vervoorn MD, Lenaers PJ, and Tamminga S 1997. Milk urea nitrogen as a tool to
590 monitor the protein nutrition of dairy cows. Journal of Dairy Science 80, 3333-3340.
591 Hristov AN, Etter RP, Ropp JK, and Grandeen KL 2004. Effect of dietary crude protein level
592 and degradability on ruminal fermentation and nitrogen utilization in lactating dairy
593 cows. Journal of Animal Science 82, 3219-3229.
594 Huhtanen P, and Hristov AN 2009. A meta-analysis of the effects of dietary protein
595 concentration and degradability on milk protein yield and milk N efficiency in dairy
596 cows. Journal of Dairy Science 92, 3222-3232.

597 Huhtanen P, Cabezas-Garcia EH, Krizsan SJ, and Shingfield KJ 2015. Evaluation of
598 between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the
599 association with nitrogen utilization and diet digestibility in lactating cows. Journal of Dairy
600 Science 98, 3182-3196.
601 Jenkins SG, Partridge ST, Stephenson TR, Farley SD, and Robbins CT 2001. Nitrogen and
602 carbon isotope fractionation between mothers, neonates, and nursing
603 offspring. Oecologia 129, 336-341.
604 Kauffman AJ, and St-Pierre NR 2001. The relationship of milk urea nitrogen to urine nitrogen
605 excretion in holstein and jersey cows1. Journal of Dairy Science 84, 2284-2294.
606 Kohn RA, Dinneen MM, and Russek-Cohen E 2005. Using blood urea nitrogen to predict
607 nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs,
608 and rats. Journal of Animal Science 83, 879-889.
609 Macko SA, and Estep ML 1984. Microbial alteration of stable nitrogen and carbon isotopic
610 compositions of organic matter. Organic Geochemistry 6, 787-790.
611 Martínez del Rio C, Wolf N, Carleton SA, and Gannes LZ 2009. Isotopic ecology ten years
612 after a call for more laboratory experiments. Biological Reviews 84, 91-111.
613 Minagawa M, and Wada E 1984. Stepwise enrichment of 1519N along food chains: further
614 evidence and the relation between δ 15 N and animal age. Geochimica et Cosmochimica
615 Acta 48, 1135-1140.
616 Pinheiro JC, and Bates DM 2000. Linear mixed-effects models: basic concepts and
617 examples. In Mixed-effects models in S and S-Plus, 3-56 (Springer, New York).
618 Phuong, H N, Friggens NC, De Boer IJM, and Schmidely P 2013. Factors affecting energy
619 and nitrogen efficiency of dairy cows: A meta-analysis. Journal of Dairy Science 96, 7245-
620 7259.
621 Poupin N, Bos C, Mariotti F, Huneau JF, Tomé D, and Fouillet H 2011. The nature of the
622 dietary protein impacts the tissue-to-diet 15N discrimination factors in laboratory rats. PloS
623 ONE 6, e28046.

624 Poupin N, Mariotti F, Huneau JF, Hermier D, and Fouillet H 2014. Natural isotopic signatures
625 of variations in body nitrogen fluxes: a compartmental model analysis. PLoS Computational
626 Biology 10, e1003865.
627 Sauvant D, and Nozière P 2016. Quantification of the main digestive processes in ruminants:
628 the equations involved in the renewed energy and protein feed evaluation
629 systems. Animal 10, 755-770.
630 Sears J, Hatch SA, and O'Brien DM 2009. Disentangling effects of growth and nutritional
631 status on seabird stable isotope ratios. Oecologia 159, 41-48.
632 Sick H, Roos N, Saggau E, Haa, K, Meyn V, Walch B, and Trugo N 1997. Amino acid
633 utilization and isotope discrimination of amino nitrogen in nitrogen metabolism of rat liverin
634 vivo. Zeitschrift für Ernährungswissenschaft 36, 340-346.
635 Sponheimer M, Robinson T, Ayliffe L, Passey B, Roeder B, Shipley L, Lopez E, Cerling T,
636 Dearing D, and Ehleringer J 2003a. An experimental study of carbon-isotope fractionation
637 between diet, hair, and feces of mammalian herbivores. Canadian Journal of Zoology 8, 871-
638 876.
639 Sponheimer M, Robinson TF, Roeder BL, Passey BH, Ayliffe LK, Cerling TE, Dearing MD,
640 and Ehleringer JR 2003b. An experimental study of nitrogen flux in llamas: is 14 N
641 preferentially excreted? Journal of Archaeological Science 30, 1649-1655.
642 Sutoh M, Obara Y, and Yoneyama, T 1993. The effects of feeding regimen and dietary
643 sucrose supplementation on natural abundance of 15N in some components of ruminal fluid
644 and plasma of sheep. Journal of Animal Science 71, 226-231.
645 Vallimont JE, Dechow CD, Daubert JM, Dekleva MW, Blum JW, Barlieb CM, Liu W, Varga
646 GA, Heinrichs AJ, and Baumrucker CR 2010. Genetic parameters of feed intake, production,
647 body weight, body condition score, and selected type traits of Holstein cows in commercial
648 tie-stall barns. Journal of Dairy Science, 93, 4892-4901.
649 Vanderklift MA, and Ponsard S 2003. Sources of variation in consumer-diet δ15N
650 enrichment: a meta-analysis. Oecologia, 136, 169-182.

651 Warinner C, and Tuross N 2010. Brief communication: Tissue isotopic enrichment associated
652 with growth depression in a pig: Implications for archaeology and ecology. American Journal
653 of Physical Anthropology 141, 486-493.
654 Wattiaux MA, and Reed JD 1995. Fractionation of nitrogen isotopes by mixed ruminal
655 bacteria. Journal of Animal Science 73, 257-266.
656 Wheadon NM, McGee M, Edwards GR, and Dewhurst RJ 2014. Plasma nitrogen isotopic
657 fractionation and feed efficiency in growing beef heifers. British Journal of Nutrition 111,
658 1705-1711.

Table 1. Variance-component estimates of N use efficiency (NUE) and N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) in ruminants when random effects of study (ID), period within-study (ID/P) and diet within-period and study (ID/P/D) were considered.

	Average value (β_0)	Estimate (σ)	95% CI (σ)	ICC[1] (%)
NUE, g/g (n= 217)	0.235±0.029			
ID		0.091	0.057-0.145	75.5
ID/P		0.030	0.018-0.051	33.6
ID/P/D		0.034	0.027-0.043	64.9
Residual[2]		0.025	0.022-0.027	
$\Delta^{15}N_{animal\text{-}diet}$, ‰ (n = 282)	3.23±0.42			
ID		1.37	0.87-2.16	84.9
ID/P		0.33	0.21-0.53	32.6
ID/P/D		0.32	0.24-0.42	45.5
Residual		0.35	0.31-0.38	

[1]Intra-class correlation coefficient: total variance explained by the tested random variables. For nested random variables as ID/P/D for instance it refers to the variance explained by the dietary treatment unexplained by the experimental period and study.

[2]Total variance (σ) unexplained by the random effect of study, period and diet (within-group variability) and including mainly the between-animal variability and experimental error.

Table 2. Mixed-effect regression models of N use efficiency in ruminants (Y) on the N isotopic fractionation (X) using either treatment means or individual values[†]

		Intercept	Slope	RSE[1]	AIC[1]	BIC[1]	Variance component estimates (σ)					
							ID		P		D	
							Intercept	Slope	Intercept	Slope	Intercept	Slope
Treatment means (n = 38)												
Random-effects:												
(Eq.3)	ID[2]	0.415*±0.057	-0.055*±0.007	0.029	-139	-132	1.9e-6	0.008				
Individual values (n = 217)												
Random-effects:												
(Eq.4)	ID[2]	0.420*±0.057	-0.050*±0.014	0.033	-1021	-1002	0.182	0.044				
(Eq.5)	ID/P[2]	0.380*±0.015	-0.042*±0.005	0.032	-1064	-1038	0.029	0.010	0.006	0.007		
(Eq.6)	ID/P/D[2$]	0.358*±0.014	-0.035*±0.005	0.022	-1117[$]	-1084[$]	0.024	0.010	1.9e-6	0.005	0.020	0.007

[†] All models were tested with random effects on the intercept, slope or both. Best models were obtained when random effects on intercept and slope were included

[1] RSE = residual standard error; AIC = Akaike information criterion; BIC = Bayesian information criterion

*: *P-value* < 0.001

[2] ID = random effect of the experimental study; ID/P = random effect of period within the experimental study; ID/P/D = random effect of diet within period and experimental study

[$] Best random structure model based on AIC/BIC criteria and the log-likelihood ratio test (*P* < 0.05). Comparisons were conducted with the maximum likelihood method and when using individual values.

Table 3. Pearson correlation coefficients between nitrogen use efficiency (NUE), nitrogen isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) and parameters related to N utilization in ruminants.

	NUE[1]	EMPU_prod[1]	EMPU_tot[1]	RDP[1]	RPB[1]	EMPS_E[1]	EMPS_N[1]	DENU[1]
$\Delta^{15}N_{animal\text{-}diet}$, ‰	-0.91	-0.76	-0.73	0.64	0.63	-0.55	-0.57	-0.66
NUE[1], g/g		0.87	0.80	-0.55	-0.57	0.43	0.53	0.61
EMPU_prod[1], g/g			0.89	-0.19	-0.19	0.10	0.13	0.19
EMPU_tot[1], g/g				-0.36	-0.34	0.16	0.25	0.21
RDP[1], g/kg DM					0.99	-0.66	-0.91	-0.89
RPB[1], g/kg DM						-0.63	-0.94	-0.93
EMPS_E[1], g/kg fOM							0.63	0.68
EMPS_N[1], g/kg RDP								0.95

[1] RDP = Rumen degradable protein; RPB = Rumen protein balance; EMPS_E = Efficiency of microbial protein synthesis according to the available energy; EMPS_N = Efficiency of microbial protein synthesis according to rumen degradable protein; DENU = Digestive efficiency N use; EMPU_prod = Efficiency of metabolizable protein use for production; EMPU_tot = Efficiency of metabolizable protein use for total net protein synthesis

Figure 1. Relationship between N use efficiency and N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) in ruminants using individual values (n = 282). a) Simple linear regression analysis (*overall relationship: NUE = 0.429 -0.058× $\Delta^{15}N_{animal\text{-}diet}$)* where open triangle = dairy cows; open circles = dairy goats; closed triangles = non-lactating sheep; closed circles = growing beef cattle*,* b) Simple linear regression for each study (n = 11; *within-study regression*), c) Simple linear regression analysis for each diet (n = 38; *within-diet regression*). Coefficients for individual regressions within-study and within-diet are presented in Figure 2.

Figure 2. Confidence intervals (95%) for the intercepts and slopes obtained from the regression of N use efficiency (Y) in ruminants on N isotopic fractionation (X). a): Within-study regression (ID#); b): Within-diet regression (ID#_T). When non-significant estimates were found, the letters *NS* appears beside the corresponding confidence interval. All slopes are numerically negative within-study (a) and 31 out of 38 within-diet (b). However, large confidence intervals are obtained because of the relative low number of observations within-study (n= 15 to 34) and within-diet (n= 3 to 16) leading to non-significant responses in 4 studies out of 11 and in 29 dietary treatments out of 38. The average slope was -0.053 and -0.046 within-study and within-diet, respectively, compared to a slope of -0.058 between studies (Fig 1).

Figure 3. Overall relationship between N use efficiency in ruminants (N retention or milk N secretion/N intake) and N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) when taking into account the random effects of the study, diet (within-study) and period (within-diet) on the intercept and slope. The resulting equation is: NUE = 0.358 (±0.014) -0.035 (±0.0050) × $\Delta^{15}N_{animal\text{-}diet}$ (RSE = 0.022). Black circles identify individual data from the only study (ID#8) using non-productive animals. Dashed lines depict 95% confidence intervals of the regression equation.

Figure 4. Simple linear regression between residuals of N use efficiency in ruminants (NUE) and N isotopic fractionation ($\Delta^{15}N_{animal\text{-}diet}$) obtained when both variables were independently adjusted for the random effects of the study, period (within-study) and diet (within-period and study). Equation: NUE = -0.024 (±0.0039) × $\Delta^{15}N_{animal\text{-}diet}$ ($P < 0.001$; $R^2 = 0.12$, RSE = 0.019).

Figure 5. Partial Least Squares (PLS) regression analysis of either a) NUE or b) $\Delta^{15}N_{animal\text{-}diet}$ on descriptors of ruminant N partitioning (Efficiency of metabolizable protein use for production [EMPU_prod]; Efficiency of metabolizable protein use for total net protein synthesis [EMPU_tot]; Rumen degradable protein [RDP; Rumen protein balance [RPB]; Efficiency of microbial protein synthesis according to the available energy [EMPS_E]; Efficiency of microbial protein synthesis according to rumen degradable protein [EMPS_N]; Digestive efficiency of N use [DENU]). Best PLS regression model kept three components (t) in both cases with Q2 values ranging from 0.83 ($\Delta^{15}N_{animal\text{-}diet}$) to 0.92 (NUE). The most important variables according to VIP (variable importance in projection) were in both cases EMPU_prod (1.17 to 1.41) and EMPU_tot (1.12 to 1.24) and are highlighted in bold type in both graphics.